

Reports

Effect of Ambient pH on Corneal Endothelial Sodium Fluxes

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Sodium fluxes across the isolated rabbit corneal endothelium were measured as a function of ambient pH and bicarbonate concentrations. At pH 7, the net sodium flux ($J_{\text{endo}}^{\text{str}} \text{ net}$) was effectively obliterated at all bicarbonate concentrations, relative to controls at pH 7.5. At pH 8, the net sodium flux was obliterated at 5 mM bicarbonate, was unchanged at 25 mM bicarbonate, and was enhanced at 40 mM bicarbonate, relative to controls at pH 7.5. The decrease in net sodium flux under these conditions relative to previously demonstrated maintenance of bicarbonate fluxes under almost identical conditions suggest that sodium and bicarbonate transverse the endothelium by different routes. In addition, changes in unidirectional fluxes, when ambient pH is decreased from 7.5 to 7, are not equal, illustrating that the fluxes probably occur via different pathways. A complex interrelationship exists between sodium, bicarbonate, and proton movement. *Invest Ophthalmol Vis Sci* 27:1274–1277, 1986

Ionic fluxes occur across the rabbit corneal endothelium, but much remains to be learned about their interactions, driving forces, and relationship to corneal fluid movement. Measurement of unidirectional bicarbonate^{1–5} and sodium^{6,7} fluxes has indicated that a net flux of both ions exist across the endothelium. The net flux of bicarbonate is dependent on ambient bicarbonate concentration,^{1,2,5} sodium concentration,⁴ and temperature,³ whereas the net flux of sodium is both bicarbonate- and temperature-dependent.³ Both fluid transport^{1,8,9} and net sodium movement⁷ are sensitive to 10^{-4} M ouabain, but 10^{-6} M ouabain has no effect on the net fluxes of either sodium or bicarbonate.³ Net bicarbonate flux is independent of pH between pH 7 and 8.⁵

It is evident that fluid transport shares some similarities with the net fluxes of both sodium and bicarbonate in its responses to environmental perturbations, but there are also differences in responses. Recently, it has been suggested that there is a Na^+/H^+ antiport at the basolateral surface of bovine tissue-cultured endothelial cells in series with a tightly-coupled sodium and bicarbonate symport system at the apical cell membrane.^{10,11} It is the intent of this paper to examine the effects of pH and bicarbonate concentration variation on transendothelial sodium fluxes.

Materials and Methods. Adult albino rabbits, 2–3 kg, were killed with an overdose of intravenous sodium pentobarbital. Animals in this study were used in accordance with the ARVO Resolution on the Use of Animals in Research. Corneas were de-epithelialized by scraping with a Gill corneal knife and mounted in sealed flux chambers, as described previously.^{2–5} Ionic fluxes were determined at 35°C on paired corneas; one for stromal to endothelial flux ($J_{\text{endo}}^{\text{str}}$) and the other for endothelial to stromal flux ($J_{\text{str}}^{\text{endo}}$). The $J_{\text{endo}}^{\text{str}}$ flux consists of an active and a passive component, while $J_{\text{str}}^{\text{endo}}$ is totally a passive flux. Because the passive component of $J_{\text{endo}}^{\text{str}}$ equals the totally passive $J_{\text{str}}^{\text{endo}}$, the values for each pair were subtracted to give a net flux ($J_{\text{endo}}^{\text{str}} - J_{\text{str}}^{\text{endo}} = J_{\text{endo}}^{\text{str}} \text{ net}$) prior to statistical analysis of the data. Each pair of corneas from the same animal was used at a specific pH and bicarbonate concentration; on any given day one pair was at pH 7.5, the other pair at either pH 7 or pH 8.

Sodium fluxes were measured by sampling of bathing solutions every 30 min over a 3 hr period as described previously,^{2–6} in one of several solutions following tracer (²²Na) equilibration for at least 1 hr. The bathing solution was Krebs-bicarbonate Ringer (concentrations in mM: NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.18; KH₂PO₄, 1.18; NaHCO₃, 25; glucose, 27.8; gassed with 3% CO₂/air; pH 7.5) with added adenosine (0.5 mM) and glutathione (0.3 mM).⁸ A pH of 7, 7.5, or 8 was used in combination with a bicarbonate concentration of 4.5, 20, or 30 mM. The 4.5 mM bicarbonate solution contained sucrose to maintain the osmolarity at 305 ± 5 mOsm, and the 30 mM bicarbonate solution had a reduced NaCl concentration also to maintain osmolarity. The pH and PCO₂ (mm Hg) of the solutions were verified using a Radiometer blood-gas analyzer immediately before placement in the sealed flux chambers. The values were: 4.5 mM, pH 7.0, PCO₂, 20; pH 7.4, PCO₂, 6; pH 8.0, PCO₂, 2; 20 mM, pH 7.0, PCO₂, 78; pH 7.45, PCO₂ 28; pH 8.0, PCO₂, 8; 30 mM, pH 7.0, PCO₂, 130; pH 7.5, PCO₂, 42; pH 8.0, PCO₂, 10. The solutions were placed in sealed syringes that were attached to the chambers. Any small changes^{2,5} in solution composition that might occur are eliminated by the solution renewal.

Table 1. Sodium fluxes across rabbit corneal endothelium as a function of ambient pH and bicarbonate concentrations

$[HCO_3^-]$	pH	n	J_{endo}^{str}	J_{str}^{endo}	J_{Net}
4.5 mM	7.0	33	10.44 ± 0.22*	11.19 ± 0.27*	-0.75 ± 0.14*
	7.5	35	11.37 ± 0.29	10.02 ± 0.34	1.35 ± 0.17
	7.5	33	12.29 ± 0.27	10.86 ± 0.32	1.43 ± 0.20
	8.0	33	11.36 ± 0.24*	11.24 ± 0.26	0.12 ± 0.11*
20 mM	7.0	32	10.62 ± 0.20	10.30 ± 0.21*	0.31 ± 0.13*
	7.5	30	11.14 ± 0.23	8.61 ± 0.26	2.53 ± 0.11
	7.5	26	12.13 ± 0.21	10.00 ± 0.24	2.14 ± 0.16
	8.0	33	11.29 ± 0.20*	8.93 ± 0.27*	2.36 ± 0.18
30 mM	7.0	28	10.25 ± 0.13*	10.23 ± 0.22*	0.02 ± 0.19*
	7.5	25	10.83 ± 0.20	9.47 ± 0.28	1.36 ± 0.22
	7.5	26	10.13 ± 0.20	8.71 ± 0.22	1.42 ± 0.13
	8.0	27	10.92 ± 0.28*	8.81 ± 0.27	2.12 ± 0.24*

Values are the mean ± SEM of n determinations and are in $\mu\text{eq} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$.

* significantly different from parallel controls at pH 7.5, $P < 0.05$ using the two sided t-test.

Results. The results from corneas run in parallel (i.e., pH 7.0 to 7.5, and 7.5 to 8.0) are shown in Table 1. Despite minor differences in the control series at each bicarbonate concentration (i.e., data at pH 7.5), the data analysis gave the same statistically significant differences whether compared within groups or whether all the control data was pooled.

The net sodium flux, (J_{endo}^{str} net), was significantly decreased at both pH 7 and 8 in a 4.5 mM bicarbonate Ringer, but was significantly decreased only at pH 7 in 20 mM and 30 mM bicarbonate Ringer. Net sodium flux was increased at pH 8 in 30 mM bicarbonate Ringer. At 4.5 mM bicarbonate concentration, the changes at pH 7 were an increase in the J_{endo}^{endo} unidirectional flux, and a decreased J_{endo}^{str} component which gave a slightly negative (stroma to endothelium) net flux. At pH 8, the change was reflected in a significant decrease in the J_{endo}^{str} component only, resulting in a significant decrease in the net flux. At 20 mM, the change at pH 7 was restricted to an increase in the J_{endo}^{endo} flux, while at pH 8 this flux decreased in both directions equally, leaving the net flux (J_{endo}^{str} net) unaltered. At 30 mM and pH 7, there was a significant decrease in the J_{endo}^{str} flux and an increased (J_{endo}^{endo}) flux relative to the data at pH 7.5. At 30 mM and pH 8 there was a stimulation of J_{endo}^{str} flux (the totally passive, J_{endo}^{endo} , flux remained constant) resulting in an increased net flux compared with pH 7.5. The net flux was effectively obliterated at pH 7 at all bicarbonate concentrations. At pH 8 the net flux was obliterated at 4.5 mM bicarbonate, was unaffected at 20 mM bicarbonate, and was enhanced at 30 mM bicarbonate.

Discussion. Both ambient pH and bicarbonate concentration influenced sodium fluxes. The effect of am-

bient pH was such that lowering pH from 7.5 to 7.0 resulted in an increased passive sodium flux (J_{endo}^{endo}) at all bicarbonate concentrations, with a simultaneous decrease in the net flux to near zero. Reduction of pH from 8.0 to 7.5, however, increased J_{endo}^{endo} only at 20 mM bicarbonate concentration. There was a substantial decrease in the J_{endo}^{str} flux at 4.5 and 30 mM when pH was decreased from 7.5 to 7.0, and an increase in the J_{endo}^{str} flux at 30 mM. Reduction of ambient pH from 8 to 7.5 increased J_{endo}^{str} at 4.5 and 20 mM, but decreased this flux at 30 mM bicarbonate.

These data were obtained at steady state following equilibration of the tissue with the ambient solution for at least 1 hr. Nevertheless, previous data on intracellular pH has shown that the gradient is sustained between the cell and the ambient solution for up to 3 hr. In a 25 mM bicarbonate solution, and at ambient pH of 7.0, the intracellular pH is 6.59; at ambient pH 7.5, the cellular pH is 7.10, and at ambient pH 8.0, cellular pH is 7.63. Thus, an almost constant difference of 0.4 pH units is maintained between the cell and the environment, with a slight decrease in the pH gradient as the ambient pH is increased.¹²

The control unidirectional sodium fluxes at pH 7.5 are not significantly different between each bicarbonate concentration. Net sodium flux is reduced, however, at very low bicarbonate concentrations.^{3,6} It would appear, therefore, that the changes noted here are primarily those induced by external pH either directly or by the influence of the changes in PCO_2 . The flux changes are assumed to be due to pH and bicarbonate changes alone and not due to alterations in PCO_2 . Previous studies have shown little influence of CO_2 concentration changes on net bicarbonate flux,⁵ which

would presumably be more responsive to ambient CO_2 changes.

It is known that external pH influences the maintenance of corneal thickness, but the limiting values (pH 6.6 and 8.3) are outside the range imposed here and, thus, the sodium flux changes do not reflect damage to the endothelium.⁹ Below a pH of 7.4, however, it was noted that the transendothelial potential fell by 50% at pH 7.⁹ The reduction in $J_{\text{endo}}^{\text{str}}$ and the increase in $J_{\text{str}}^{\text{endo}}$ found with a decrease in ambient pH from 7.5 to 7.0 is consistent with the expected decrease in transendothelial negativity under the same ambient pH reduction. A reduction of the transendothelial potential (aqueous side negative) would reduce the driving force on the passive movement of sodium across the endothelium.

The data indicate a marked effect of decreased ambient pH (increased H^+ concentration) from 7.5 to 7.0 on net sodium flux, with a decrease in all bicarbonate concentrations. In addition, at 4.5 mM bicarbonate a decrease from pH 7.5 to 7 reversed the direction of the net flux. These decreases in net flux are coupled with an increase in passive sodium permeability ($J_{\text{str}}^{\text{endo}}$) and may reflect an alteration in the paracellular pathways; passive bicarbonate flux also increases as the ambient pH becomes more acidified,⁵ confirming that a passive membrane event is altered, although there is always a net bicarbonate flux oriented towards the endothelial-facing side even at 5 mM bicarbonate.^{4,6} The flux $J_{\text{str}}^{\text{endo}}$ is increased to a greater extent than $J_{\text{endo}}^{\text{str}}$ is decreased (Table 1), indicating that the sodium fluxes occur across the endothelium by different pathways.

The decrease in the $J_{\text{endo}}^{\text{str}}$ component at 4.5 or 30 mM bicarbonate when the pH is changed from 7.5 to 7 cannot be attributed to an alteration of the proposed Na^+/H^+ antiport in the basal cell surface¹⁰ given that the transplasma membrane H^+ gradient increases slightly under the incubation conditions used in these experiments as it does at 25 mM bicarbonate.¹² An external pH decrease, therefore, must either affect the exit of sodium from the cell, rather than its entry, due to an effect on the proposed coupled sodium:bicarbonate symport in the apical cell membrane,¹⁰ or by causing a change in other transendothelial pathways. Given such a symport system, one would predict that the movement of bicarbonate from cell to apical bathing solution would conceivably be enhanced because the existing bicarbonate would combine with the protons to form carbonic acid and hence CO_2 and water, thereby creating a larger gradient for cell to solution movement of bicarbonate. Such an event should enhance sodium exit from the cell rather than be an impediment. The changes in pH in the present experiments, however, occurred on both sides of the membrane, and a combination of a decrease in sodium entry

through a $\text{Na}:\text{H}$ exchange system and an increase in sodium exit via the apically located symport could lead to a change in $J_{\text{endo}}^{\text{str}}$ that would be dependent upon which translocation system was changed the most.

The increase in $J_{\text{endo}}^{\text{str}}$ and net flux when the ambient pH is raised from 7.5 to 8 in 30 mM bicarbonate should enhance the $\text{Na}:\text{HCO}_3^-$ symport mediated exit of sodium from the cell; but, since changes were made in the bathing solution on both sides of the membrane, the resultant fluxes would also depend on the relative effects of the pH change on different ion systems. The increase in pH from 7.5 to 8 also increased the net flux at 30 mM, had no effect at 20 mM, and decreased net flux at 5 mM; these effects are contrary to those found with bicarbonate fluxes under similar conditions,⁵ and may reflect a dissociation of net sodium flux from net bicarbonate flux across the endothelium. This data would argue (including the effects at pH 7 where transendothelial potential changes may also influence the net flux of sodium) that sodium and bicarbonate transfer across the endothelium are not always linked. This data supports an alternative model for endothelial ion movement in which sodium is transferred via extracellular, rather than intracellular, pathways.¹¹

There is a strong dependence of sodium fluxes on ambient pH, suggesting that a Na^+/H^+ exchange mechanism is present despite difficulties relating the changes to the suggested system.¹⁰ The present data also suggest that the interaction between sodium, bicarbonate, and protons is more complex than suggested from electrophysiological data,¹⁰ including modulation either of sodium entry into, or exit from, the endothelial cell by external H^+ , or modulation via paracellular pathways. Certainly, deviations from the normal ambient pH of 7.5 at 4.5 mM bicarbonate are more deleterious than at other bicarbonate concentrations, indicating that bicarbonate plays an important role in sustaining transendothelial sodium movement. The complexity of the interrelationships is illustrated by the decreased sodium transport when either the proton concentration is enhanced or reduced in 5 mM bicarbonate bathing solutions, and opposite effects (i.e., a decrease at low pH and an enhancement at high pH) in 30 mM bicarbonate bathing solutions.

The present data indicate that decreases of ambient pH (7.5 to 7, or 8 to 7.5), for the most part, increase passive sodium movement across the endothelium. Any Na^+/H^+ exchange mechanism at the basolateral membrane must play a small role in the net movement of Na^+ across the endothelium. Perhaps the role of the latter system (Na^+/H^+) is in cell volume or pH regulation. The present data do not support the concept of a strong link, such as a $\text{Na}^+:\text{HCO}_3^-$ symport being involved in transendothelial sodium movement. The different effects of changes in ambient pH on both bicar-

bonate⁵ and sodium fluxes (Table 1) also argue against a strong link between these ions. The data suggest that the movement of sodium may be more strongly influenced by the transendothelial potential (and hence current flows) developed by the net transfer of bicarbonate. This supports the concept that, while the transendothelial net flux of bicarbonate is via a cellular pathway, the net movement of sodium may occur via a paracellular pathway as suggested by one of the models presented by Fischbarg et al.¹¹

Key words: corneal endothelium, sodium fluxes, ambient pH effects

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Epithelial Ion Transport in Rabbit Corneas Following Myopic Keratomileusis

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In isolated rabbit corneas that had undergone lamellar keratectomy or myopic keratomileusis, the stimulation of chloride transport by 10^{-5} M epinephrine was completely inhibited at 1 week following surgery. At 28 days following surgery, both groups responded to 10^{-7} M epinephrine. The response to 10^{-5} M amphotericin B was normal both at 1 week and at 28 days following surgery. We conclude that, although the Na^+K^+ pump was not affected by the lamellar keratectomy and cryolathing, that either the epithelial β receptors and/or the cAMP pathway were temporarily inhibited for at least 1 week following surgery. A lamellar keratectomy, therefore, can have an adverse effect on the epithelial transport system of the corneal epithelium even though the epithelium may appear normal clinically. *Invest Ophthalmol Vis Sci* 27:1277-1280, 1986

In myopic keratomileusis, a resected lamellar disc from the central cornea is frozen and thinned to effect a flattening of the cornea and correction of the myopic refractive error. The thickness of stromal tissue re-

moved is dependent on the correction and typically ranges from 0.10-0.20 mm.

Following clinical myopic keratomileusis, the central corneal thickness is frequently greater than that predicted by the carving parameters. As it is certain that a given amount of tissue has been resected, such a finding may be explained by an increase in the state of hydration of the cornea. This may result from a temporary diminution in the physiologic capacity of the corneal endothelium, changes in the ground substance, rupture of cells by the freezing process with subsequent release of cellular contents, and the marked polymorphonuclear influx observed histologically following cryorefractive surgery. Any of these may account for an increase in the water content of the cornea.

It is also known that the corneal epithelium plays both a passive and an active role in helping maintain corneal deturgescence.¹⁻⁴ This is mediated in large part by an active chloride transport system that has been